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DOI: https://doi.org/10.1021/acs.orglett.5b01602

Organic LETTERS

Letter

pubs.acs.org/OrgLett

Total Synthesis of the Glycosylated Macrolide Antibiotic Fidaxomicin

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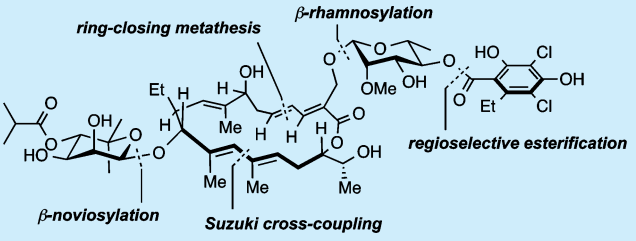
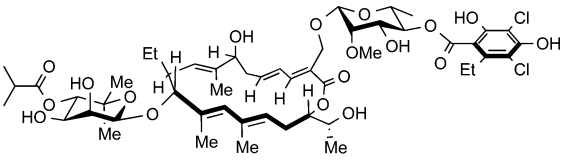
Supporting Information

ABSTRACT: The first enantioselective total synthesis of fidaxomicin, also known as tiacumicin B or lipiarmycin A3, is reported. This novel glycosylated macrolide antibiotic is used in the clinic for the treatment of *Clostridium difficile* infections. Key features of the synthesis involve a rapid and high-yielding access to the noviose, rhamnose, and orsellinic acid precursors; the first example of a β-selective noviosylation; an effective Suzuki coupling of highly functionalized substrates; and a ring-closing metathesis reaction of a noviosylated dienoate precursor. Careful selection of protecting groups allowed for a complete deprotection yielding totally synthetic fidaxomicin.



Antibacterial resistance constitutes a growing concern in public health for many societies all over the world,[1] and many antibiotics have become less effective if not ineffective against many pathogens.[2] This is particularly striking given that the last discovery of a new class of antibiotics dates back to the 1980s. Tuberculosis (TB) has become a threatening example among the diseases that evolved to be resistant to common antibiotic therapy. In 2012 alone, out of 8.7 million new cases of TB, 450 000 patients were infected by multidrug resistant-TB.[1] With increased frequency, frontline antibiotics (such as rifampicin or isoniazid) fail in therapeutic treatment. All these issues call for new antibiotics to fill this gap.[3] To this end, we became interested in fidaxomicin (**1**, tiacumicin B, lipiarmycin A3),[4] which inhibits RNA polymerase in bacteria and shows high activity against drug resistant strains of *Mycobacterium tuberculosis*.[5] Fidaxomicin (**1**) was FDA-approved in 2011 for the treatment of *Clostridium difficile* infections involved in nosocomial (hospital acquired) diarrhea. Even though advantageous for the therapy of CDI, the low bioavailability of fidaxomicin (**1**) in the plasma by oral application prohibits its use as a therapeutic against systemic diseases such as TB.

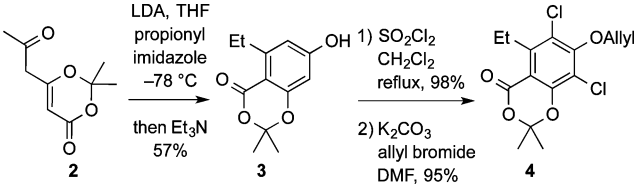
In addition to the interesting bioactivity, the 18-membered macrolactone constitutes an architecturally complex synthetic target featuring multiple stereogenic centers and a high degree of unsaturation. The central macrolide is β-linked to a unique D-noviose and D-rhamose, which is complemented with a dichloro homoorselinic acid unit. Despite the fact that fidaxomicin (**1**) has been isolated in the 1970s and is clinically used to date, no total synthesis of a member of this class has been reported in the literature.[7] Coincidentally, syntheses of the core aglycon have been recently published back-to-back-to-back by Altmann et al.,[8] Zhu et al.,[9] and our group.[10] Furthermore, synthetic studies have been performed on 4-OMe D-noviose[11] carbohydrates[12] and a resorcinol unit.[13] The challenges of fidaxomicin (**1**) concerning its total synthesis reside in (1) rapid access to the carbohydrate and orsellinate building blocks; (2) two challenging β-selective glycosylations (cf. the *cis*-1,2-diol β-mannose problem);[14] and (3) a suitable protecting group strategy allowing for selective deprotection. In this study, we report on the first total synthesis of fidaxomicin (**1**). Our highly convergent synthetic route assembles five main fragments by a β-selective noviosylation, a Suzuki cross-coupling, a ring-closing metathesis (RCM), and a β-rhamnosylation.



Fidaxomicin (**1**, tiacumicin B, lipiarmycin A3)

The synthesis commenced with the preparation of the carbohydrate and orsellinic acid precursors. For the preparation of the protected resorcylate **4**, we chose the biomimetic aromatization strategy established by Barrett et al.[15] (Scheme 1). Therefore, the dianion of the known keto dioxinone **2**[16,17] was reacted with freshly prepared propionyl imidazole[18] to furnish the corresponding diketo dioxinone. The subsequent aromatization under basic conditions directly yielded the resorcylate **3** (57%). Chlorination with sulfuryl chloride[19,20]

Scheme 1. Synthesis of the Protected Resorcylate 4



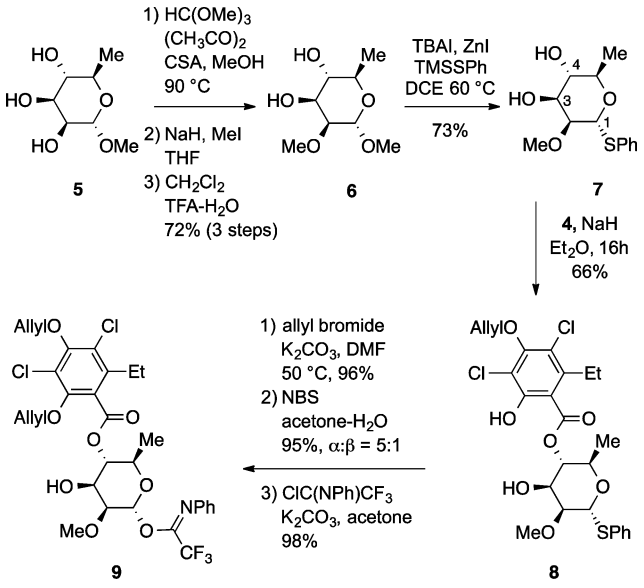
Received: June 1, 2015
Published: June 30, 2015

DOI: 10.1021/acs.orglett.5b01602
Org. Lett. 2015, 17, 3514–3517

proceeded in quantitative yield, and the allylation gave rise to the protected homoorsellinic acid **4** in excellent yield (95%).

The preparation of the rhamnose unit **9** originated in the known D-rhamnoside **5**[21] (Scheme 2), which was synthesized

Scheme 2. Synthesis of the Rhamnosyl Donor 9



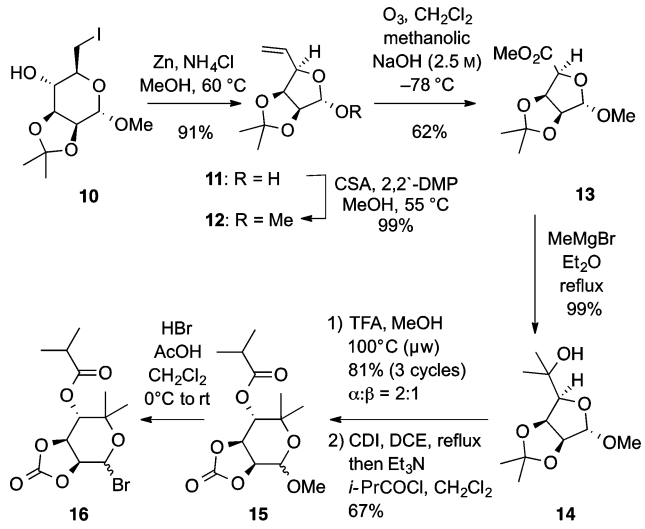
from the corresponding methyl-α-D-mannopyrannoside by a Garegg Samuelsson iodination and subsequent Pd-catalyzed hydrogenation.[22,23] Butane-2,3-diacetal protection of the *trans*-3,4-hydroxy groups allowed for the selective installation of the methyl group at O-2, and subsequent deprotection furnished the diol **6** in 72% yield over three steps. Next, the thiophenyl group was installed by ZnI_2 mediated trans-acetalization with TMSSPh to afford the thioglycoside **7**.[24]

To our delight, the coupling reaction between the resorcylate **4** and the diol **7** proceeded with excellent regioselectivity at O-4 to yield the ester **8** (66%). Interestingly, the O-3 esterified regioisomer was initially formed exclusively and the ester migration occurred with a prolonged reaction time to give rise to the desired product **8**. Next, allylation of the phenolic OH group (96%) and hydrolysis of the thioacetal with NBS in acetone/H_2O 10:1 was performed with good yields and selectivity (95%, α/β = 5:1). Finally, the lactol was functionalized with *N*-phenyl trifluoroacetimidoyl chloride to give the rhamnosyl donor **9**.[25] The switch of the leaving group was crucial in order to achieve good yields as well as anomeric selectivity in the rhamnosylation step (*vide infra*).

The known 6-deoxy-6-iodopyranoside **10**[26] served as starting material for the preparation of the novioside unit (Scheme 3). First, a Vasella ring contraction by a modified procedure[27,28] furnished the olefinic furanose **11** (91%). Treatment of lactol **11** with CSA in methanol and 2,2′-dimethoxypropane gave the furanoside **12** without cleavage of the acetonide. Ozonolysis of the terminal olefin using Marshall's protocol[29] in methanolic sodium hydroxide gave direct access to the ester **13** (62%). The major side product in this oxidative cleavage was the corresponding aldehyde (11%), which could be transformed to the desired ester under the same reaction conditions.

Next, the *gem*-dimethyl groups were introduced using MeMgBr to afford the tertiary alcohol **14** in excellent yield (99%). The alcohol **14** was then treated with TFA in methanol

Scheme 3. Synthesis of Noviosylbromide 16



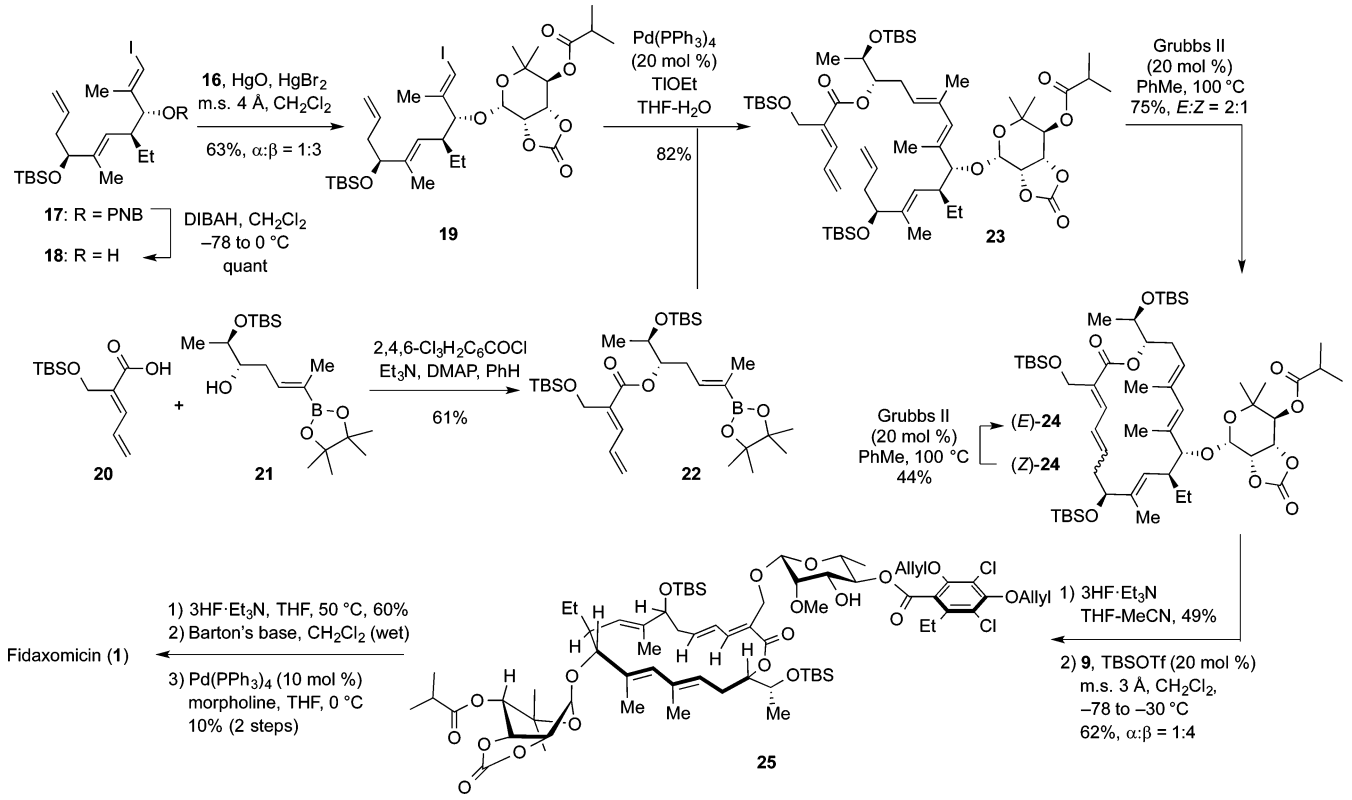
at high temperature, first to hydrolyze the acetonide and second to isomerize the furanoside to the pyranoside. As the equilibrium of the 5- vs 6-membered ring was found to be only 1:2 in favor of the desired pyranoside, the furanoside was recovered and resubmitted twice to the same reaction conditions. In this way, the unfunctionalized novioside was obtained as an inseparable anomeric mixture in good overall yield (81%, α/β = 2:1). Based on the previous studies in the area of β-rhamnosylation,[14] we opted for introducing the electron-withdrawing 2,3-carbonate protecting group with CDI, which was followed by esterification with isobutyryl chloride to yield the two separable anomers of **15** (67% over two steps). Finally, the acetal **15** (either epimer) was converted to the desired glycosyl bromide donor **16** using HBr in acetic acid. Due to the instability of glycosyl bromides, the intermediate was used in the next step without purification.

At this point we aimed for the assembly of the three building blocks **9**, **16**, and the previously prepared aglycon.[10] Even though we achieved satisfactory β-rhamnosylation on the primary alcohol (not shown), the β-noviosylation on the complete macrolide proved exceptionally challenging. Tedious experimentation using different glycosidation methods consistently resulted only in α-selective glycosylation on the protected macrolide.

Hypothesizing that the alcohol in the rigid ring system is poorly accessible for an attack from the sterically hindered face of the glycoside (cf. the β-mannose problem),[14] we sought to introduce the noviose unit on a linear, more flexible intermediate at an early stage. Therefore, we prepared the alcohol **18** by reductive cleavage of the PNB group of fragment **17** (99%, Scheme 4), which constituted an intermediate in our aglycon synthesis.[10] We were pleased to find that the glycosylation with the sterically demanding, yet more flexible secondary alcohol **18**, using Helferich's conditions,[30] furnished the noviosylated fragment **19** with a good α/β ratio (63%, α/β = 1:3, 48% of β-anomer isolated). The relative configuration of the β-glycosidic linkage was assigned by NMR studies (NOESY). To the best of our knowledge, this experiment constitutes the first example of a β-selective noviosylation that has been reported in the literature. To assemble the remaining parts of the macrocycle, fragment **22** was prepared from the known precursor **21**[8] by a Yamaguchi esterification (61%) with

DOI: 10.1021/acs.orglett.5b01602
Org. Lett. 2015, 17, 3514–3517

Scheme 4. Completion of the Total Synthesis



the dienoic acid **20**.[10] The following Suzuki cross-coupling of the boronate **22** and iodide **19** was highly effective and furnished product **23** in good yield. Fortunately, the basic conditions ($Pd(PPh_3)_4$, TlOEt in THF–H_2O), first used by Altmann and Glaus in their aglycon synthesis,[8] did not affect the base labile carbonate and ester moiety, which can be attributed to the very short reaction time (less than 30 min).[31] The subsequent ring-closing metathesis reaction by treatment of the linear fragment **23** with the second generation Grubbs catalyst (20 mol %) for 1 h at 100 °C gave the macrolide with an (*E*/*Z*) ratio of 2:1 in 75% yield (54% of *E*-**24**). The chromatographic separation of the *E*/*Z* isomers, allowed for a subsequent recycling of the (*Z*) isomer. As a result, the overall yield of (*E*)-**24** in this transformation was increased to 63%. Selective deprotection of the primary TBS ether was achieved with trihydrogenfluoride triethylamine in moderate yield (49%). As anticipated, the *β*-selective installation of the rhamnosyl side chain turned out to be challenging.[14] After an extensive screening, we were able to obtain high *β*-selectivity and a good yield with the trifluoroacetimidate donor **9** in its $^4C_1$ conformation (α/β = 1:4, estimated by ^1H NMR analysis of the crude reaction mixture, 62% of **25**).[25,14e] Conveniently, the protection of the O-3 hydroxy group was unnecessary.

The final task for the completion of the total synthesis was the deprotection of the TBS, carbonate, and allyl groups. First, the two TBS groups were removed by treatment with trihydrogenfluoride triethylamine at 50 °C (60%). The following carbonate deprotection proved to be troublesome due to competitive isobutyrate ester hydrolysis. Nevertheless, Barton's base in wet CH_2Cl_2 gave satisfactory results (good conversion and purity, as judged by NMR analysis). Finally, Pd-catalyzed allyl-deprotection and two-stage purification (preparative TLC followed by HPLC) gave fully synthetic fidaxomicin (**1**, 10% over two steps). The observed overall yield for these transformations can be explained by difficulties in the purification of the final product on a small scale. The identity of the synthetic compound was confirmed by coinjection of synthetic and authentic material on reversed-phase HPLC. Interestingly, as the synthetic material contained residual formate from the HPLC purification, the ^1H NMR spectrum did at first not fully match with the spectra of the natural product. Again, by mixing equimolar amounts of synthetic and authentic samples, the ^1H NMR spectrum clearly confirmed the identity of the synthetic material to authentic fidaxomicin (**1**).

In this letter, we report the first, enantioselective total synthesis of the glycosylated macrolide antibiotic fidaxomicin (**1**), which has been isolated over 40 years ago and constitutes a clinically used drug. Key features of the synthetic route include (1) a rapid access to the rhamnosyl side chain, (2) the first *β*-selective noviosylation followed by Suzuki cross-coupling and ring-closing metathesis of complex noviosylated precursors, and (3) a *β*-selective rhamnosylation. In particular, the stereoselective installment of both 1,2-*cis* diols in the *β*-linked carbohydrate units constitutes a notable feature of the approach. This successful total synthesis contributes to a detailed understanding of the chemistry of fidaxomicin and paves the way for the generation of analogs addressing some of the shortcoming of the natural product.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data, NMR spectra and HPLC chromatogram of fidaxomicin (**1**). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01602.

DOI: 10.1021/acs.orglett.5b01602
Org. Lett. 2015, 17, 3514–3517

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge partial financial support by the NCCR Molecular Systems Engineering, the Latsis Prize (to K.G.), and the Novartis Early Career Award (to K.G.). We thank Dominik Lotter and Reto Witzig at University of Basel for skillful technical support and Priv.-Doz. Dr. D. Häussinger (University of Basel) for assistance with NMR spectroscopy.

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DOI: 10.1021/acs.orglett.5b01602
Org. Lett. 2015, 17, 3514−3517